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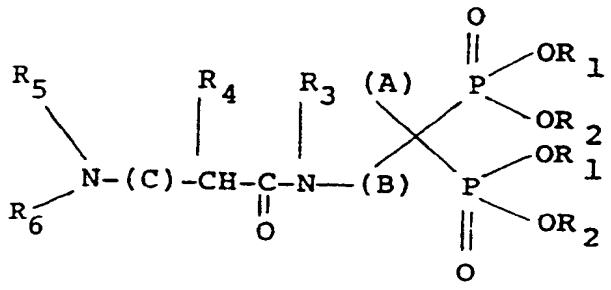
(54) Title: GEM-DIPHOSPHONIC ACIDS, A PROCESS FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM



(I)

(57) Abstract

Diphosphonic acids of formula (I), wherein R_1 and R_2 are hydrogen or C_1-C_4 alkyl; (A) is hydrogen, halogen, hydroxy or C_1-C_{12} alkyl; (B) is a bond, a C_1-C_8 alkylene chain, a cycloalkylalkylene chain, an alkylene chain substituted by cyclohexyl or cyclopentyl groups, or an aralkyl chain, an alkyl chain containing an heteroatom (O, S or N-CH_3) or an ureido residue -$(CH_2)_{n1}$-NHCONH -$(CH_2)_n$- with n ranging from 1 to 5, R_3 is hydrogen, C_1-C_9 alkyl, C_3-C_6 cycloalkyl, benzyl, phenyl or p-methoxybenzyl; (C) is C_1-C_5 alkyl, phenyl or an aralkyl chain; R_4 is hydrogen, C_1-C_4 alkyl or an amino group optionally substituted by alkyl, phenyl, benzyl, p-methoxybenzyl, acyl, amminoacidic or peptide groups; R_5 and R_6 are 2-haloethyl or together form a 1-aziridinyl residue, are useful as antitumor agent.

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GEM-DIPHOSPHONIC ACIDS, A PROCESS FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM.

The present invention relates to diphosphonic acids having antitumor activity, a process for the preparation thereof and pharmaceutical compositions containing them.

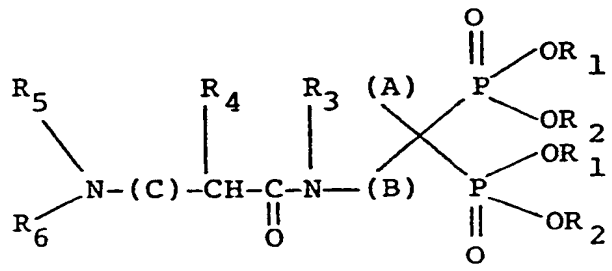
Gem-diphosphonic acids and the salts thereof are known and used in the treatment of osteoporosis and bone resorption (EP 96.931, EP 252.504, BE 896.453, BE 903.519, DE 3.016.289, DE 3.540.150, DE 2.534.391). Moreover, diphosphonic acid esters having pesticide activity are disclosed in US-Pat. 3.906.062. However, no compounds described in the above mentioned patents have been reported to have intrinsic antitumor activity.

DE 3.425.812 discloses 1,1-diphosphonic acid derivatives having a bis[(haloalkyl)amino]phenyl residue as agents useful for the treatment of bone tumours. In fact, beside having the bone tropism characteristic of diphosphonic acids, they also have the typical cytotoxic activity of molecules bearing dialkylating functions.

It has now been found that diphosphonic acid derivatives characterized by the presence of a bond which can be physiologically hydrolyzed, connecting the diphosphonic derivative with a dialkylating residue, have, compared with the above cited compounds, advantageous antitumor and antimetastatic properties, which could not be predicted on the basis of their chemical structure and of the presumed bioconversion thereof

into the separate components (diphosphonic derivative and alkylating derivative).

The present invention, therefore, provides compounds of formula (I)

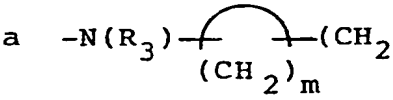
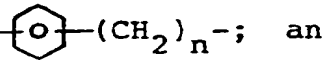


(I)

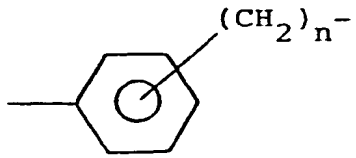
wherein :

R_1 and R_2, which can be the same or different, are hydrogen or C_1-C_4 alkyl;

(A) is hydrogen, halogen (chlorine, bromine or iodine), hydroxy, straight or branched C_1-C_{12} alkyl;

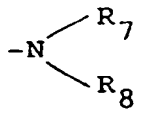
(B) is a covalent bond, a straight or branched C_1-C_8 alkylene or, together with the adjacent nitrogen atom, a group of formula  , which is optionally gem 1,1; 1,2; 1,3 or 1,4 disubstituted; an ortho, meta or para -substituted aralkyl of formula  ; an alkylene chain containing at least one hetero-atom of formula $-[CH(CH_3)]_p-(CH_2)_{n1}-X-(CH_2)_n-$; m is the integer 5 or 6; n and n_1 are an integer from 1 to 5; p is zero or 1 and X is O, S, $\overset{|}{N}-CH_3$ or the ureido group -NH-CO-NH-; R_3 is hydrogen, straight or branched C_1-C_9 alkyl, C_3-C_6 cycloalkyl, benzyl, phenyl or p-methoxybenzyl;

(C) is straight or branched C_1-C_5 alkyl, phenyl, an aralkyl chain of formula

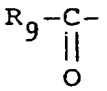


in which n is as above defined;

R_4 is hydrogen, straight or branched C_1-C_4 alkyl, or it is a group of formula



in which R_7 and R_8, which are the same or different, are hydrogen, straight or branched C_1-C_6 alkyl, phenyl, benzyl, p-methoxybenzyl, or one of R_7 and R_8 is as above defined and the other one is a group of formula



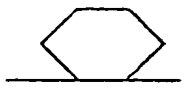
in which R_9 is hydrogen, straight or branched C_1-C_4 alkyl, phenyl, benzyl, p-methoxyphenyl, straight or branched C_1-C_4 alkoxy, halo-C_1-C_4-alkoxy; R_5 and R_6 are haloethyl (2-chloroethyl, 2-bromoethyl, 2-iodoethyl) or R_5 and R_6, together with the nitrogen atom to which they are bound, are a 1-aziridinyl residue of formula

The present invention also includes racemic and diastereoisomeric mixtures as well as the single enantiomers and diastereoisomers of the compounds of formula (I).

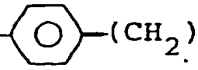
The present invention further comprises the pharmaceutically acceptable salts of the compounds of formula (I), for example with inorganic bases, such as the salts with alkali metals (e.g. sodium or potassium) or with alkaline-earth metals (e.g. calcium or magnesium), or the ammonium salts; the salts with organic bases, such as methylamine, ethylamine, propylamine,

isopropylamine, butylamine, t-butylamine, dimethylamine, diethylamine, diethanolamine, trimethylamine, triethylamine, piperidine, pyridine, picoline, dicyclohexylamine; the organic acid addition salts, such as: formate, acetate, trifluoroacetate, maleate, fumarate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate salts; the inorganic acid addition salts, such as hydrochloride, hydrobromide, sulphate, hydrogen sulphate, phosphate salts; or the salts with amino acids, such as aspartates, glutamates or the salts with lysine or arginine.

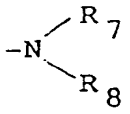
C_1-C_4 Alkyl includes methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl or isobutyl; particularly preferred are methyl and ethyl. The C_1-C_{12} alkyl group can be, beside the meanings above precised for C_1-C_4 alkyl, n-pentyl, n-hexyl, n-decyl and the like; particularly preferred are methyl and ethyl. The alkylene chain (B) is preferably $-(CH_2)_q-$, wherein q is an integer from 2 to 5, $-\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}-(CH_2)_r-$ wherein r is an integer from 2 to 5, or one of the groups of formulae



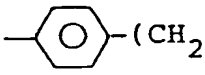
wherein s is an integer from 1 to 4.

R_3 is preferably hydrogen or methyl, whilst (C) is preferably benzyl or 

When (C) is benzyl, R_4 is preferably a group of formula

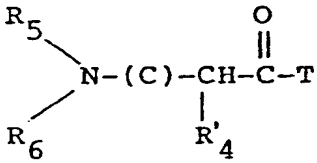


wherein R_7 and R_8 are preferably hydrogen, or one of them is R_9-CO- wherein R_9 is hydrogen, methyl, tert-butoxy, trichloromethoxy, (2,2,2)trichloroethoxy, benzyloxy, ethoxy.

When (C) is a residue of formula  , R_4 is preferably hydrogen.

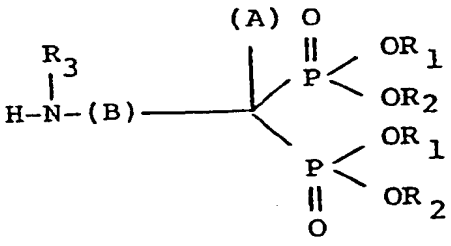
R_5 and R_6 are preferably 2-chloroethyl.

The compounds according to formula (I) are prepared by reaction of a compound of formula (II)



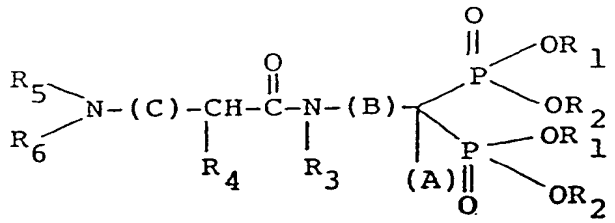
(II)

wherein R_5, R_6 and (C) are as above defined and R'_4 is the same as R_4 or a group which can be transformed into R_4 by removal of any protecting groups present, T is hydroxy or a group which activates the carboxylic function with a compound of formula (III)



(III)

wherein R_1, R_2, R_3, (A) and (B) are as above defined, to give a compound of formula (Ia)



(Ia)

wherein R_1, R_2, R_3, R_4, R_5, R_6, (C), (B) and (A) are as above defined, which compound can in its turn be transformed into a compound of formula (I) by means of well-known reactions for the selective elimination of the protecting groups, or by alkylating or acylating the amino groups, and the like.

When in the reaction of compounds (III) with compounds (II) the latter are used in form of carboxylic acids (T = OH), the reaction is generally carried out in the presence of a condensing agent such as N,N'-dicyclohexylcarbodiimide, N-cyclohexyl-N'-morpholinoethylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide; N,N'-carbonyl-bis(imidazole); phosphorus oxychloride; phosphorus trichloride; thionyl chloride; oxalyl chloride; ethyl chloroformate; isobutyl chloroformate; morpholinoethylisonitrile and the like. Examples of activated forms of carboxylic acids, according to formula (III), are acid halides, symmetrical or mixed anhydrides (e.g. with methanesulfonic, acetic, isobutyric, pivalic, trichloroacetic acids); activated amides (e.g. with imidazole or triazole); acylazide; activated esters (e.g. p-nitrophenyl ester, methoxymethyl ester, 2,4-dinitrophenyl ester, pentachlorophenyl ester, hydroxysuccinimido ester, 1-hydroxy-2-(1H)-pyridone ester, 1-hydroxybenzotriazole ester) and the like. The reaction can be carried out in the presence of an inorganic base, such as an alkali

carbonate or hydrogen carbonate, an alkaline or alkaline-earth hydroxide or an organic base, such as triethylamine, tributylamine, pyridine, dimethylaminopyridine, N-alkylmorpholine, N,N-dialkyl-aniline and the like.

The reaction can be carried out at a temperature ranging from -40°C to the reflux temperature of the solvent, preferably using a slight molar excess of compound (II) to compound (III), in a solvent such as water, pyridine or N,N-dimethylformamide or mixtures thereof.

The reaction temperature preferably ranges from -10°C to room temperature and the reaction time ranges from 1 to 48 hours, but generally the reaction is complete within 2-12 hours.

Alternatively, the mixed anhydrides or the acid chlorides of the compounds of formula (II) can be reacted with the compounds of formula (III) in heterogeneous phase, in a solvent such as an ester, e.g. methyl or ethyl formates; ethyl, methyl or isopropyl acetates; or in a halogenated solvent such as dichloromethane or chloroform, or in a straight or cyclic ether such as dioxane, tetrahydrofuran, ethyl ether, tert-butylmethyl ether and mixtures thereof. Temperature conditions and reaction times are the same as above reported.

Compounds of general formula (II) are known compounds, which are commercially available and/or can be prepared by means of conventional methods, such as those described in : J. Med. Chem. 24, 1304, (1981); CA 51: 8066d, (1957); BE 905,974; CA 104: 141897 (1986);

J. Med. Chem. 7, 468, (1964); J. Med. Chem. 6, 85, (1963); Cancer Chem. Rep. 50, 685, (1966); J. Med. Chem. 21, 16, (1977); J. Org. Chem. 26, 1554, (1961); J. Org. Chem. 26, 1674, (1961); CA 64: 10267g, (1966); J. Chem. Soc., 2994, (1960); Biochem. Pharmacol. 11, 847, (1962); Biochem. Pharmacol. 12, 833, (1963); CA 73: 131293c, (1970); Biochem. Pharmacol. 5, 192, (1960).

Compounds of general formula (III) are also known and/or can be prepared according to known methods. See, for instance, EP 96,931, EP 252,504, BE 903,519, DE 3,016,289, EP 224,751, DE 2,534,391, EP 197,478.

If desired, compounds of general formula (I) wherein R_1 and R_2 are different from hydrogen, can optionally be transformed into the corresponding gem-diphosphonic acids by treatment with a molar excess of trialkylsilyl-chloride, -iodide or -bromide in a halogenated solvent such as dichloromethane, 1,2-dichloroethane, 1,1,2-trichloroethane and the like. Trimethylsilyl iodide is preferably used.

The reaction times ranges from a few minutes to 72 hours; the reaction temperatures range from 0°C to the solvent's reflux temperature; preferred reaction conditions are those according to J. Org. Chem: 28, 2975-78, (1963).

The removal of the secondary- or primary-amine protecting groups optionally present in the compounds of general formula (I) can be carried out according to well-known techniques, particularly those used in peptide synthesis.

The compounds of the invention have high cytotoxic

activity against tumour cells, as it can be evidenced by means of "in vitro" tests carried out, for instance, according to the procedure described by M.P. Hacker, Cancer Res. 45, 4748, (1985). The ID_{50} (i.e. the compound dose which can inhibit by 50% the growth of "in vitro" cultured murine and human tumour cells of both solid and hematic tumors) of the compounds of the invention were found to be comprised from 0.1 to 5 µg/ml of culture medium. Under these test conditions, the compounds of the invention: N-{4-[bis(2-chloroethyl)amino]phenyl-(L)-alanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid monohydrochloride and N-{4-[bis(2-chloroethyl)amino]phenyl-butirroyl}-4-amino-1-hydroxy-butane-1,1-diphosphonic acid have an ID_{50} of 0.1 µg/ml and 0.5 µg/ml, respectively, against murine leukemia L1210.

"In vitro" studies on the compound of the invention N-{4-[bis(2-chloroethyl)amino]phenyl-(L)-alanyl}-4-amino-1-hydroxy-butane-1,1-diphosphonic, using different human tumor cell lines (for example fibrosarcoma HT 1080, osteosarcoma H2-OS etc.) showed ID_{50} ranging from 13.6 to 16.5 µg/ml; the substance appears to be less cytotoxic than the related starting material 4-amino-1-hydroxy-1,1-diphosphonic acid, whose ID_{50} range from 2.7 to 8.2 µ/ml.

The same compound, when tested "in vivo" by i.v. administration (on days 2 - 6 - 9 - 13 after tumor implantation) in rats bearing Walker B mammary carcinoma (implanted on day 0 intramuscolarly 10^6 cells) causes complete regression of tumor growth combined with normalization of tumor induced

hypercalcemia. Under the same experimental conditions, the related starting diphosphonate (i.e. 4-amino-1-hydroxy-butane-1,1-diphosphonic acid) even if endowed with higher "in vitro" cytotoxicicy, is surprisingly ineffective in retarding tumor growth, also when administered at its maximum tolerated dosage.

The compounds of the invention are characterized by a low acute toxicity and are well tolerated by the animals.

The compounds of the invention have a high therapeutical index, in light of the low toxicity and the effective antitumor activity thereof. Moreover, the high water-solubility of the compounds of the present invention allows the easy preparation of parenteral and oral pharmaceutical forms.

The compounds of formula (I), when administered to humans and animals affected with tumours which can be treated with alkylating agents, at doses ranging from 1 mg to 1.2 g/m^2 body area, can induce the regression of the above mentioned tumoral forms.

The effective dosage for the compounds of the invention can be determined by the expert clinicians according to conventional methods. The relationship between the dosages used for various animal species and those for humans (on the basis of mg/m^2 body area) is described by Freireich, E.J., et al., Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey and Man, Cancer Chemother. Rep., 50, n. 4, 219-244, May 1966.

Nevertheless, the patient will be administered generally with doses from 1 to 1,200 mg/kg body weight of

the compounds of the invention, with a dosage regimen which will vary depending on various factors, which are well known to the expert clinicians.

Some compounds of the invention may have such an high toxicity, or such an unfavourable therapeutical index, so as to be unsuited for an antitumor treatment in the patients. Nevertheless, those parameters can easily be determined by means of conventional toxycological tests, such as acute and subacute DL_{50} in mouse. Of course, those compounds which turn out to be toxic will be avoided.

The compounds of the invention will be used in the treatment of those tumours which can be treated with alkylating agents.

Particularly, multiple myeloma, osteosarcoma, bone-metastasis; breast, ovarian and testis carcinomas can also be advantageously treated.

Moreover, the compounds of the invention can advantageously be used in the therapy of other solid and hematic neoplasias, such as lymphomas and leukemias in humans and animals, according to treatment protocols which can be easily determined by those skilled in the art.

The compounds of the invention are preferably administered by the intravenous or intraarterial routes, even though other administration forms can be envisaged in particular cases.

The pharmaceutical forms which can be used for parenteral administration include sterile aqueous solutions or sterile powders for the extemporary preparation of solutions, as well as oily preparations for in-

tramuscular or intraperitoneal administrations.

Other useful pharmaceutical forms are syrups or similar liquid forms, as well as solid forms such as tablets, capsules and the like.

The following examples illustrate the invention in more detail.

EXAMPLE 1

a) A solution of bis(tert-butoxy)carbonate (450 mg) in tetrahydrofuran (THF; 4 ml) is quickly added to a solution of 4-[bis(2-chloroethyl)amino]-(L)-phenylalanine (300 mg) in THF (10 ml) and 1N NaOH (1 ml).

The resulting mixture is stirred at room temperature for 2 hours, adjusting pH to about 9 by repeated additions of 1N NaOH. Then the solvent is removed under vacuum and the residue is partitioned between water (3 ml) and ethyl ether (5 ml).

The organic phase is discharged and the aqueous one is acidified with HCl and repeatedly extracted with ethyl ether. The combined organic extracts are dried over Na_2SO_4 and evaporated to dryness under vacuum, to obtain a foamy residue of N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)phenylalanine (290 mg). NMR ($CDCl_3$, TMS) δ = 1.4 (s, 9H); 3.1 (m, 2H); 3.65 (m 8H); 4.55 (m, 1H); 4.99 (d, 1H); 6.62 (d, 2H); 7.12 (d, 2H); 8.1 (s, 1H).

b) N-Hydroxysuccinimide (173 mg) and morpholino-ethylisonitrile (0.133 ml) are added to a solution of the compound from step a) (290 mg). The mixture is stirred at room temperature for one hour, then concentrated to dryness under vacuum. The residue is taken up into 2N HCl (5 ml) and repeatedly extracted with ethyl

ether (3 x 5 ml). The organic phases are collected, washed with 5% aqueous $NaHCO_3$, with water (5 ml) and then dried over Na_2SO_4. Upon removing the solvent under vacuum, an oily residue is obtained which is crystallized from an ethyl ether - isopropyl ether mixture to give 260 mg of N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanine hydroxysuccinimido ester, melting point 140-143°C;

NMR (TMS, $CDCl_3$) δ = 1.45 (s, 9H); 2.85 (s, 4H); 3.15 (m, 2H); 3.69 (m, 8H); 4.9 (m, 1H); 6.61 (d, 2H); 7,15 (d, 2H).

c) A solution of the obtained activated ester (50 mg) in dimethylformamide (DMF, 2.5 ml) is slowly dropped into a solution of 5-amino-1-hydroxypentane-1,1-diphosphonic acid (21.8 mg) dissolved in a mixture of water (2.5 ml), 1N NaOH (0.331 ml) and DMF (2 ml). DMF is added to the reaction mixture simultaneously to the ester solution, until complete dissolution of the reagents is obtained (2.5 ml).

The obtained slightly opalescent solution is centrifuged; the supernatant liquid is collected and concentrated under reduced pressure. The residue is diluted with DMF (5 ml) to separate a precipitate which is collected by centrifugation and washed with ethyl acetate, yielding 35 mg of N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid trisodium salt, m.p. >260°C.

NMR (TMS, D_2O): δ 1.3 (s, 9H); 1.4-1.7 (m, 4H); 1.8-2.05 (m, 2H); 2.62-3.1 (m, 2H); 3.2 (t, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H).

EXAMPLE 2

Following the same procedure as described in Example 1, by reacting N'-(tertbutoxycarbonyl)-4-[bis-(2-chloroethyl)amino]-(L)-phenylalanine hydroxysuccinimido ester with the appropriate aminoalkyl-1-hydroxy-1,1-diphosphonic acids, the following compounds are obtained:

N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-1-hydroxybutane-1,1-diphosphonic acid (trisodium salt),

NMR (D_2O, TMS): δ = 1.3 (s, 9H); 1.6 (m, 2H), 1.92 (m, 2H); 2.62 ÷ 3.1 (m, 2H); 3.2 (t, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-amino-1-hydroxypropane-1,1-diphosphonic acid (trisodium salt),

NMR (D_2O, TMS): δ = 1.32 (s, 9H); 2.1 (m, 2H); 2.65 ÷ 3.1 (m, 2H); 3.21 (t, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-6-amino-1-hydroxyhexane-1,1-diphosphonic acid (trisodium salt),

NMR (D_2O, TMS): δ = 1.3 (s, 9H); 1.5 (m, 2H); 1.6 (m, 4H); 1.9 (m, 2H); 2.65 ÷ 3.1 (m, 2H); 3.20 (t, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino)]-(L)-phenylalanyl}-4-amino-1-hydroxypentane-1,1-diphosphonic acid (trisodium salt),

NMR (D_2O, TMS): δ = 1.32 (s, 9H); 1.4 (d, 3H); 1.7 (m, 2H); 1.9 (m, 2H); 2.6 ÷ 3.05 (m, 2H); 3.15 (d, 1H); 3.75 (s, 8H); 4.2 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H);

N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-(2-aminocyclopent-1-yl)-1-hydroxypropane-1,1-diphosphonic acid (trisodium salt),
NMR (D_2O, TMS): δ = 1.3 (s, 9H); 1.42÷1.75 (m, 9H); 1.9 (m, 2H); 2.62 ÷ 3.1 (m, 2H); 3.3 (m, 1H); 3.75 (s, 8H);4.2 (t, 1H);6.85 (d, 2H); 7.15 (d, 2H);
N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-pentamethylene-1-hydroxybutane-1,1-diphosphonic acid (trisodium salt),
NMR (D_2O, TMS): δ = 1.3 (s, 9H): 1.55 (m, 10H); 1.6 (m, 2H); 1.9 (m, 2H); 2.62 ÷ 3.1 (m. 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.85 (d, 2H);7.15 (d, 2H);
N-methyl-N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid (trisodium salt),
NMR (D_2O, TMS): δ = 1.3 (s, 9H); 1.55 (m, 4H); 1.9 (m, 2H); 2.1 (s, 3H); 2.62 ÷ 3.1 (m, 2H); 3.2 (t, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H);
N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino)]-(L)-phenylalanyl}-4-amino-4,4-tetramethylene-1-hydroxybutane-1,1-diphosphonic acid (trisodium salt),
NMR (D_2O, TMS): δ = 1.3 (s, 9H); 1.5 (m, 8H); 1.6 (m, 2H); 1.9 (m, 2H); 2.62 ÷ 3.1 (m, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H).

EXAMPLE 3

Following the same procedure as described in Example 1b), by reacting 4-[4-[bis(2-chloroethyl)amino]-phenyl]butyric acid (300 mg) with N-hydroxysuccinimide (245 mg) and N-morpholinoethylisonitrile (0.18 ml), 4-[4-[bis(2-chloroethyl)amino]phenyl]butyric acid N-hydroxysuccinimido ester (360 mg) is prepared, m.p. 80-

82°C.

Following the same procedure as described in Example 1c), the above hydroxysuccinimido ester (112 mg) is reacted with 5-amino-1-hydroxypentane-1,1-diphosphonic acid (61.2 mg) to obtain N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid trisodium salt (80 mg), m.p. >260°C.

NMR (TMS, D_2O): δ = 1.4-1.7 (m, 4H); 1.8 ÷ 2.05 (m, 4H); 2.15 (t, 2H); 2.55 (t, 2H); 3.18 (t, 2H); 3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H).

HPLC : Partisfere^R C_{18}, 150 x 4.6 mm; 0.025 M sodium heptanesulfonate in water/acetonitrile/dioxane 70:20:10, pH ~2.5 with H_3PO_4; flow : 1.3 ml/min; λ = 255 nm. Retention time : 6.86'.

EXAMPLE 4

Following the procedure described in Example 3, by reacting N-4-[4-[bis(2-chloroethyl)amino]phenyl]butyric acid hydroxysuccinimido ester with the appropriate aminoalkyl-1-hydroxy-1,1-diphosphonic acids, the following compounds are obtained:

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-amino-1-hydroxybutane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.7 ÷ 2.05 (m, 6H); 2.25 (t, 2H); 2.55 (t, 2H); 3.18 (t, 2H); 3.75 (s, 8H); 6.85 (d, 2H); 7.2 (d, 2H);

HPLC : Partisfere^R C_{18}, 150 x 4.6 mm; 0.025 M sodium heptanesulfonate in water/acetonitrile/dioxane 70:20:10, pH ~ 2.5 with H_3PO_4; flow 1.3 ml/min.; λ = 255 nm. Retention time : 5.96'.

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-3-

amino-1-hydroxypropane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.8 ÷ 2.05 (m, 4H); 2.25 (t, 2H); 2.55 (t, 2H);3.18 (t, 2H);3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-6-amino-1-hydroxyhexane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.4 (m, 2H); 1.6 (m, 4H); 1.9 (m, 4H); 2.25 (t, 2H); 2.55 (t, 2H);3.18 (t, 2H);3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-amino-1-hydroxypentane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.4 (d, 3H); 1.7 (m, 2H); 1.9 (m, 4H); 2.25 (t, 2H); 2.55 (t, 2H);3.18 (d, 1H);3.75 (s, 8H); 6.85 (d, 2H); 7.2 (d, 2H);

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-3-(2-aminocyclopent-1-yl)-1-hydroxypropane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.42 ÷ 1.75 (m, 9H); 1.9 (m, 4H); 2.25 (t, 2H); 2.55 (t, 2H);3.2 (m, 1H);3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-amino-4,4-pentamethylene-1-hydroxybutane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.55 (m, 10H); 1.6 (m, 2H); 1.9 (m, 4H); 2.15 (t, 2H); 2.55 (t, 2H);3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H);

N-methyl-N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid

(trisodium salt),

NMR (TMS, D_2O): δ = 1.55 (m, 4H); 1.9 (m, 4H); 2.1 (s, 3H); 2.15 (t, 2H); 2.55 (t, 2H); 3.18 (t, 2H); 3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H);

N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-amino-4,4-tetramethylene-1-hydroxybutane-1,1-diphosphonic acid (trisodium salt),

NMR (TMS, D_2O): δ = 1.5 (m, 8H); 1.7 ÷ 2.05 (m, 6H); 2.15 (t, 2H); 2.55 (t, 2H); 3.75 (s, 8H); 6.8 (d, 2H); 7.15 (d, 2H).

EXAMPLE 5

A solution of N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid trisodium salt (35 mg) in methanol saturated with hydrochloric acid (2 ml) is heated to 40-50°C for 4 hours, then the solution is concentrated to reduced volume and the precipitated sodium chloride is filtered. The filtrate is evaporated to dryness and the residue is triturated with acetone and filtered. After recrystallization from ethanol/ethyl ether, N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid monohydrochloride (25 mg) is obtained, m.p. > 260°C, $[\alpha]_D$ = + 7.9 (c = 2, methanol), NMR (TMS, D_2O): 1.25 ÷ 1.40 (m, 2H), 1.42 ÷ 1.72 (m, 2H); 1.8 ÷ 2.06 (m, 2H); 2.9 ÷ 3.2 (m, 4H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (t, 1H); 7.35 (s, 4H).

HPLC : Partisfere^R C_{18}, 150 x 4.6 mm; 0.025 M sodium heptanesulfonate in water/acetonitrile/dioxane 70:20:10, pH ~ 2.5 with H_3PO_4; flow 1.3 ml/min.; $\nearrow$ =

255 nm. Retention time : 4.61'.

EXAMPLE 6

Following the same procedure as described in Example 5, by reacting the trisodium salts of the acids described in Example 2, the following acid monohydrochloride salts are obtained :

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-1-hydroxybutane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 1.6 (m, 2H); 1.92 (m, 2H); 3.15 (m, 4H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H);

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-amino-1-hydroxypropane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 2,1 (m, 2H); 3,15 (m, 4H); 3,65 (t, 4H); 4,0 (t, 4H); 4,1 (m, 1H); 7,35 (s, 4H);

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-6-amino-1-hydroxyhexane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 1.5 (m, 2H); 1.6 (m, 4H); 1.9 (m, 2H); 3.15 (m, 4H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H);

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-1-hydroxypentane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 1.4 (d, 3H); 1.7 (m, 2H); 1.9 (m, 2H); 3.15 (m, 3H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H);

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-(2-aminocyclopent-1-yl)-1-hydroxypropane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 1.5 (m, 8H); 1.6 (m, 2H); 1.9 (m, 2H); 3.15 (t, 2H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H);

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-pentamethylene-1-hydroxybutane-1,1-diphosphonic

acid: NMR (D_2O, TMS): δ = 1.45 (m, 10H); 1.6 (m, 2H); 1.9 (m, 2H); 3.15 (t, 2H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H);

N-methyl-N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 1.55 (m, 4H); 1.9 (m, 2H); 2.1 (s, 3H); 3.05 (t, 2H); 3.15 (t, 2H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H);

N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-tetramethylene-1-hydroxybutane-1,1-diphosphonic acid: NMR (D_2O, TMS): δ = 1.5 (m, 8H); 1.6 (m, 2H); 1.9 (m, 2H); 3.15 (t, 2H); 3.65 (t, 4H); 4.0 (t, 4H);4.1 (m, 1H); 7.35 (s, 4H).

EXAMPLE 7

A solution of 4-[bis(2-chloroethyl)amino]-(L)-phenylalanine (150 mg) in formic acid (1.82 ml) and acetic anhydride (0.64 ml) is stirred at room temperature for 3 hours. The reaction mixture is concentrated to reduced volume under reduced pressure and partitioned between water (3 ml) and ethyl acetate (2 x 50 ml). The organic phase is dried over sodium sulphate and the solvent is evaporated under vacuum, to give N-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanine (160 mg) as a yellow foam, $[\alpha]_D$ = +67° (C = 2, ethanol).

NMR ($CDCl_3$, TMS): = 2.9 ÷ 3.1 (m, 2H); 3.6 (m, 8H); 4.68 ÷ 4.79 (m, 1H); 6.5 (m, 2H+1H); 6.69 (d, 2H); 8.1 (s, 1H).

Following the same procedure as described in Example 1b) and 1c), this compound is transformed into the hydroxysuccinimido ester (50 mg) and then is reacted with 4-amino-1-hydroxybutane-1,1-diphosphonic acid

(24.1 mg), to obtain N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-1-hydroxybutane-1,1-diphosphonic acid trisodium salt (32 mg); $[\alpha]_D$ = +7.5° (c = 2, water)

NMR (D_2O, TMS): δ = 1.7 ÷ 2.0 (m, 4H); 2.9 ÷ 3.1 (m, 2H); 3.2 (m, 2H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H).

HPLC : Partisfere^R C_{18}, 150 x 4.6 mm; 0.025 M sodium heptanesulfonate in water/acetonitrile/dioxane 70:20:10, pH ~ 2.5 with H_3PO_4; flow 1.3 ml/min.; λ = 255 nm. Retention time : 2.9'.

EXAMPLE 8

Following the same procedure as described in Example 1c), by reacting N-formyl-4-[bis(2-chloroethyl)-amino]-(L)-phenylalanine hydroxysuccinimido ester with the appropriate aminoalkyl-1-hydroxy-1,1-diphosphonic acids, the following compounds are obtained :

- N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid trisodium salt;

NMR (D_2O, TMS): δ = 1.4 ÷ 1.6 (m, 4H); 1.92 (m, 2H); 2.9 ÷ 3.1 (m, 2H); 3.2 (m, 2H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H);7.15 (d, 2H); 8.05 (s, 1H);

- N-{N'-formyl-[4-bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-amino-1-hydroxypropane-1,1-diphosphonic acid trisodium salt;

NMR (D_2O, TMS): δ = 2,1 (m, 2H); 2,9 ÷ 3,1 (m, 2H); 3,2 (m, 2H); 3,75 (s, 8H); 4,55 (t, 1H); 6,85 (d, 2H); 7,15 (d, 2H); 8,05 (s, 1H);

- N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-6-amino-1-hydroxyhexane-1,1-diphosphonic acid

trisodium salt;
NMR (D_2O, TMS): δ = 1.5 (m, 2H); 1.6 (m, 4H); 1.9 (m, 2H); 2.9 ÷ 3.1 (m, 2H); 3.2 (m, 2H); 3.75 (s, 8H); 4.2 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H);

- N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-1-hydroxypentane-1,1-diphosphonic acid trisodium salt;
NMR (D_2O, TMS): δ = 1.4 (d, 3H); 1.7 (m, 2H); 1.9 (m, 2H); 2.9 ÷ 3.1 (m, 2H); 3.2 (m, 2H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H);

- N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-(2-aminocyclopent-1-yl)-1-hydroxypropane-1,1-diphosphonic acid trisodium salt;
NMR (D_2O, TMS): δ = 1.42 ÷ 1.75 (m, 9H): 1.9 (m, 2H); 2.9 ÷ 3.1 (m, 2H); 3.3 (m, 1H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H);

- N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-tetramethylene-1-hydroxybutane-1,1-diphosphonic acid trisodium salt;
NMR (D_2O, TMS): δ = 1.5 (m, 8H); 1.6 (m, 2H); 1.9 (m, 2H); 2.9 ÷ 3.1 (m, 2H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H);

- N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-pentamethylene-1-hydroxybutane-1,1-diphosphonic acid trisodium salt;
NMR (D_2O, TMS): δ = 1.45 (m, 10H); 1.6 (m, 2H); 1.9 (m, 2H); 2.9 ÷ 3.1 (m, 2H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H);

- N-methyl-N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid trisodium salt;

NMR (D_2O, TMS): δ = 1.55 (m, 4H); 1.9 (m, 2H); 2.1 (s, 3H); 2.9 ÷ 3.1 (m, 2H); 3.2 (m, 2H); 3.75 (s, 8H); 4.55 (t, 1H); 6.85 (d, 2H); 7.15 (d, 2H); 8.05 (s, 1H).

EXAMPLE 9

A solution of N-{N'-formyl-4-[bis(2-chloroethyl)amino]-(L)phenylalanyl}-4-amino-1-hydroxybutane-1,1-diphosphonic acid trisodium salt (32 mg) in methanol saturated with hydrochloric acid (1 ml) is heated to 40-50°C for 4 hours, then the solution is concentrated to reduced volume and the precipitated sodium chloride is filtered. The filtrate is evaporated to dryness and the residue is triturated with acetone and filtered. After recrystallization from ethanol/ethyl ether, N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-1-hydroxybutane-1,1-diphosphonic acid monohydrochloride (25 mg) is obtained, m.p. > 260°C; $[\alpha]_D$ = + 7.8 (c = 2, methanol).

NMR (D_2O, TMS): δ = 1.6 (m, 2H); 1.92 (m, 2H); 3.15 (m, 4H); 3.65 (t, 4H); 4.0 (t, 4H); 4.1 (m, 1H); 7.35 (s, 4H).

HPLC : Partisfere^R C_{18}, 150 x 4.6 mm; 0.025 M sodium heptanesulfonate in water/acetonitrile/dioxane 70:20:10, pH ~ 2.5 with H_3PO_4; flow 1.3 ml/min.; λ = 255 nm. Retention time : 4.24'.

EXAMPLE 10

A solution of 4-[4-[bis(2-chloroethyl)amino]phenyl]butyric acid N-hydroxysuccinimido ester (73 mg) in DMF (1 ml) is dropped at room temperature into a stirred solution of 4-aminobutane-1,1-diphosphonic acid tetraethyl ester (52 mg) in DMF/water 5/1 (0.6 ml).

After 3 hours, the mixture is concentrated under

reduced pressure and partitioned between a 5% sodium hydrogen carbonate solution (5 ml) and ethyl acetate (5 ml). After separation of the organic phase, the aqueous phase is further extracted with ethyl acetate (2 x 5 ml) and discarded; the combined organic extracts are dried over sodium sulphate and solvent is evaporated off under reduced pressure, to yield N-[4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl]-4-aminobutane-1,1-diphosphonic acid tetraethyl ester (90 mg) as a clear oil;

NMR ($CDCl_3$, TMS): δ = 1.35 (t, 12H); 1.90 (t, 2H); 2.2 (t, 2H); 2.0 ÷ 2.5 (m, 5H); 2.55 (t, 2H); 3.45 (q, 2H); 3.65 (m, 8H); 4.20 (m, 8H); 6.51 (t, 1H); 6.61 (d, 2H); 7.1 (d, 2H).

EXAMPLE 11

Under a nitrogen atmosphere, a solution of iodotrimethylsilane (92 µl) in anhydrous dichloromethane (1 ml) is dropped at 0°C into a solution of N-{4-[4-[bis(2-chloroethyl)amino]phenylbutyroyl}-4-aminobutane-1,1-diphosphonic acid tetraethyl ester (72 mg) in anhydrous dichloromethane (1 ml). The mixture is stirred for 2 hours at 0°C, then it is allowed to room temperature and subsequently treated with methanol (1 ml). After 15 minutes, the solvent is evaporated off under reduced pressure and the residue is dissolved in water (2 ml), treated with 1N NaOH (0.228 ml) and extracted with ethyl acetate (2 x 2 ml). The organic extracts are discarded. The aqueous phase is diluted with DMF to yield a white solid which is recovered by centrifugation, to give N-{4-[4-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-aminobutane-1,1-dipho-

sphonic acid disodium salt (45 mg);

NMR (D_2O, TMS): δ = 1.9 (m, 6H); 2.25 (t, 2H); 2.55 (t, 2H); 3.38 (m, 2H); 3.72 (s, 8H); 6.85 (d, 2H); 7.2 (d, 2H).

EXAMPLE 12

Following the same procedure as described in Example 10, N-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)-amino]-(L)-phenylalanine-N-hydroxysuccinimido ester (87,5 mg) is reacted with 4-aminobutane-1,1-diphosphonic acid tetraethyl ester (50 mg) to obtain N-{N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-aminobutane-1,1-diphosphonic acid tetraethyl ester (87 mg) as a clear oil.

NMR ($CDCl_3$, TMS): δ = 1.38 (m, 21H); 2.2 (m, 5H); 3.45 (m, 4H); 3.65 (m, 8H); 4.18 (m, 8H); 5.05 (m, 1H); 6.51 (t, 1H); 6.61 (d, 2H); 7.1 (d, 2H).

EXAMPLE 13

A solution of N-{N'-(tert-butoxycarbonyl)-4-[bis-(2-chloroethyl)amino]-(L)-phenylalanyl}-4-aminobutane-1,1-diphosphonic acid tetraethyl ester (59 mg) in 10% HCl (1 ml) is heated to 45°C for one hour, then evaporated to dryness under reduced pressure. The residue is suspended in ethyl ether (2 ml) and recovered by centrifugation, to give N-{[4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-aminobutane-1,1-diphosphonic acid tetraethyl ester dihydrochloride (45 mg);

NMP (TMS, D_2O): δ = 1.22 (t, 12H); 1.95 (m, 4H); 3.2 (m, 2H); 3.4 (m, 2H); 3.7 (t, 4H); 3.95 (m, 12H); 4.1 (t, 1H); 7.38 (s, 4H).

EXAMPLE 14

Under a nitrogen atmosphere, a solution of iodo-

trimethylsilane (134 µl) in anhydrous dichloromethane (1 ml) is dropped into a stirred solution of N-[N'-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl]-4-aminobutane-1,1-diphosphonic acid tetraethyl ester (93 mg) in anhydrous dichloromethane (1 ml), cooled to 0°C.

The mixture is stirred for 2 hours, allowing it to warm to room temperature, then it is treated with methanol (1 ml) . After 15 minutes, solvent is evaporated off under reduced pressure, the residue is dissolved in water, treated with 1N NaOH (0.254 ml) and extracted with ethyl acetate (2 x 3 ml). The organic extracts are discarded. The aqueous phase is diluted with DMF to precipitate a solid, which is recovered by centrifugation and washed with ethyl acetate.

45 mg of N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-aminobutane-1,1-diphosphonic acid disodium salt are obtained,

NMR (D_2O, TMS): δ = 1.95 (m, 4H); 2.9 (m, 2H); 3.4 (m, 2H); 3.65 (m, 1H); 3.75 (s, 8H); 6.88 (d, 2H); 7.18 (d, 2H).

EXAMPLE 15

Following the same procedures as described in Examples 12 and 13, using N-(tert-butoxycarbonyl)-4-[bis(2-chloroethyl)amino]-(L)-phenylalanine hydroxysuccinimido ester and the appropriate tetraalkylesters of aminoalkyl-1,1-diphosphonic acids the following compounds are prepared:

- N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-aminopropane-1,1-diphosphonic acid tetraethylester dihydrochloride;

- N-[4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-aminopentane-1,1-diphosphonic acid tetraethylester dihydrochloride;
- N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-6-aminohexane-1,1-diphosphonic acid tetraethylester dihydrochloride;
- N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-aminopentane-1,1-diphosphonic acid tetraethylester dihydrochloride;
- N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-3-(2-aminocyclopent-1-yl)propane-1,1-diphosphonic acid tetraethylester dihydrochloride;
- N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-tetramethylene-butane-1,1-diphosphonic acid tetraethylester dihydrochloride;
- N-{4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-4-amino-4,4-pentamethylene-butane-1,1-diphosphonic acid tetraethylester dihydrochloride;
- N-methyl-N-4-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-aminopentane-1,1-diphosphonic acid tetraethylester dihydrochloride.

EXAMPLE 16

Following the same procedures as described in Example 1a) and 1b), 3-[bis(2-chloroethyl)amino]-(L)-phenylalanine (J. Med. Chem., 6, 85, (1963)) is transformed into N'-(tert-butoxycarbonyl)-3-[bis(2-chloroethyl)amino]-(L)-phenylalanine hydroxysuccinimido ester, which is reacted with 5-amino-1-hydroxypentane-1,1-diphosphonic acid following the procedure described in Example 1c), to give N-{N'-(tert-butoxycarbonyl)-3-[bis(2-chloroethyl)amino)-(L)-phenylalanyl}-5-amino-1-

hydroxypentane-1,1-diphosphonic acid trisodium salt.

This compound is subsequently transformed into N-{3-[bis(2-chloroethyl)amino]-(L)-phenylalanyl}-5-amino-1-hydroxypentane-1,1-diphosphonic acid dihydrochloride, following the procedure described in Example 5.

EXAMPLE 17

Following the procedure described in Example 1a) and 1b), N^6,N^6-bis(2-chloroethyl)-(DL)-lysine (J. Med. Chem. 7, 468, (1964)) is transformed into N^2-(tert-butoxycarbonyl)-N^6,N^6-bis(2-chloroethyl)-(DL)-lysine hydroxysuccinimido ester which is reacted with 4-amino-1-hydroxybutane-1,1-diphosphonic acid, following the procedure described in Example 1c), to give N-[N^2-(tert-butoxycarbonyl)-N^6,N^6-bis(2-chloroethyl)-(DL)-lysinyl]-4-amino-1-hydroxybutane-1,1-diphosphonic acid trisodium salt, which is in its turn transformed into N-[N^6,N^6-bis(2-chloroethyl)-(DL)-lysinyl]-4-amino-1-hydroxybutane-1,1-diphosphonic acid dihydrochloride, following the procedure described in Example 5.

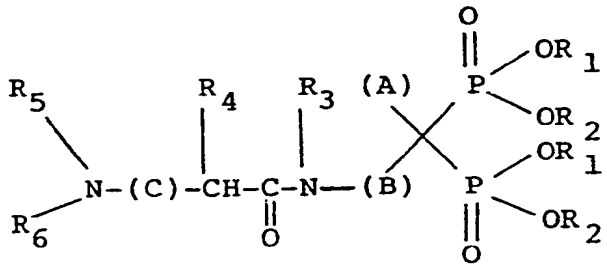
EXAMPLE 18

Following the procedure described in Example 1b), by reacting 4-[2-[bis(2-chloroethyl)amino]phenyl]butyric acid (J. Org. Chem. 26, 1554, (1961)) with N-hydroxysuccinimide and N-morpholinoethylisonitrile, 4-[2-[bis(2-chloroethyl)amino]phenyl]butyric acid N-hydroxysuccinimido ester is prepared. Said N-hydroxysuccinimido ester is reacted with 4-aminobutane-1,1-diphosphonic acid tetraethyl ester, according to the procedure described in Example 10, to give N-{4- [2-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-aminobutane-1,1-diphosphonic acid tetraethyl ester, which is reacted with

iodotrimethylsilane, following the procedure described in Example 11, to obtain N-{4-[2-[bis(2-chloroethyl)amino]phenyl]butyroyl}-4-aminobutane-1,1-diphosphonic acid disodium salt.

CLAIMS

1. Compounds of formula (I)



(I)

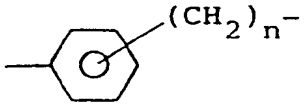
wherein :

R_1 and R_2, which can be the same or different, are hydrogen or C_1-C_4 alkyl;

(A) is hydrogen, halogen (chlorine, bromine or iodine), hydroxy, straight or branched C_1-C_{12} alkyl;

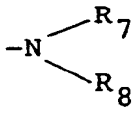
(B) is a covalent bond, a straight or branched C_1-C_8 alkylene or, together with the adjacent nitrogen atom, a group of formula $-N(R_3)-\langle\ \rangle-(CH_2)_n-$, which is optionally gem(1,1); 1,2; 1,3 or 1,4 disubstituted; an ortho, meta or para - substituted aralkyl of formula $-N(R_3)-C_6H_4-(CH_2)_n-$; an alkylene chain containing at least one hetero-atom of formula $-[CH(CH_3)]_p-(CH_2)_{n1}-X-(CH_2)_n-$, m is the integer 5 or 6; n and n_1 are an integer 1 to 5; p is zero or 1 and X is O, S, $N-CH_3$ or the ureido group -NH-CO-NH-; R_3 is hydrogen, straight or branched C_1-C_9 alkyl, C_3-C_6 cycloalkyl, benzyl, phenyl or p-methoxybenzyl;

(C) is straight or branched C_1-C_5 alkyl, phenyl, an aralkyl chain of formula

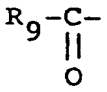


in which n is as above defined;

R_4 is hydrogen, straight or branched C_1-C_4 alkyl, or it is a group of formula



in which R_7 and R_8, which are the same or different, are hydrogen, straight or branched C_1-C_6 alkyl, phenyl, benzyl, p-methoxybenzyl, or one of R_7 and R_8 is as above defined and the other one is a group of formula



in which R_9 is hydrogen, straight or branched C_1-C_4 alkyl, phenyl, benzyl, p-methoxyphenyl, straight or branched C_1-C_4 alkoxy, halo-C_1-C_4-alkoxy; R_5 and R_6 are haloethyl (2-chloroethyl, 2-bromoethyl, 2-iodoethyl) or R_5 and R_6, together with the nitrogen atom to which they are bound, are a 1-aziridinyl residue of formula

and isomers, diastereisomers and pharmaceutically acceptable salts thereof.

2. Compounds as claimed in claim 1, wherein A is hydroxy.

3. Compounds as claimed in claim 1, wherein A is hydrogen.

4. Compounds as claimed in any one of claims 1 to 3, wherein R_1 and R_2 are hydrogen.

5. Compounds as claimed in any one of claims 1 to 3,

wherein R_1 and R_2 are C_1-C_4 alkyl.

6. Compounds as claimed in any one of claims 1 to 5, wherein (B) is a C_2-C_5 alkylene chain and R_3 is hydrogen.

7. Compounds as claimed in any one of claims 1 to 5, wherein (B) is a chain of formula

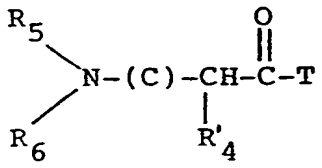
wherein s is from 1 to 4.

8. Compounds as claimed in any one of claims 1 to 7, wherein (C) is phenyl or benzyl and R_5 and R_6 are 2-chloroethyl.

9. Compounds as claimed in claim 8, wherein (C) is benzyl and R_4 is amino, t-butoxycarbonylamino, formylamino, acetylamino, benzyloxycarbonylamino or ethoxycarbonylamino.

10. Compounds as claimed in claim 8, wherein (C) is phenetyl and R_4 is hydrogen.

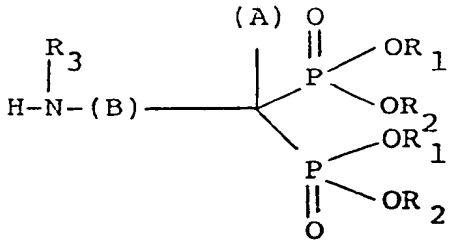
11. A process for the preparation of compounds of formula (I), which process comprises reacting a compound of general formula (II)



(II)

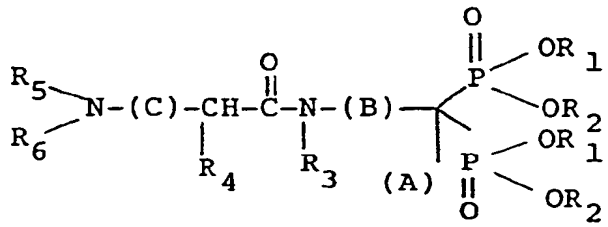
wherein R_5, R_6 and (C) are as above defined and R'_4 is the same as R_4 or a group which can be transformed into R_4 by removal of protecting groups possibly present, T is hydroxy or a carboxy-activating group,

with a compound of formula (III)



(III)

wherein R_1, R_2, R_3, (A) and (B) are as above defined, to give a compound of formula (Ia)



wherein R_1, R_2, R_3, R_4, R_5, R_6, (C), (B) and (A) are as above defined, which compound can in its turn be transformed into a compound of formula (I) by selective elimination of the protecting groups, or by alkylating or acylating the amino groups.

12. Pharmaceutical compositions having anitumor activity, containing as the active ingredient one compound of formula (I) in mixture with a non toxic carrier.

13. The use of a compound of formula (I) for the preparation of an antitumor medicament.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/01710

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 F 9/38, 9/564, A 61 K 31/66

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 F

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	DE, A1, 3425812 (HENKEL KGAA) 16 January 1986, see the whole document --	1-13
A	Chemical Abstracts, volume 109, no. 17, 24 October 1988, (Columbus, Ohio, US), Pool, Beatrice L. et al.: "In vivo and in vitro investigations on biological effects of aromatic bis-(2-chloroethyl)amino-bisphosphonic acids, new agents proposed for chemotherapy of bone tumors; cytostatic activity in rat osteosarcoma; toxicity and genotoxicity in liver and bone marrow; mutagenicity in S. typhimurium", & Invest. New Drugs 1988, 6(2) 67-78 --	1-13

* Special categories of cited documents:[10]

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"P" document published prior to the international filing date but later than the priority date claimed

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th December 1990	21 JAN 1991
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	MISS I. TAZELAAR

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/EP 90/01710

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The members are as contained in the European Patent Office EDP file on 28/11/90
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A1- 3425812	16/01/86	EP-A-B- 0170896	12/02/86
		JP-A- 61063687	01/04/86
		US-A- 4608368	26/08/86

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